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An Analysis of the Abundance, Diversity and Patchiness of Terrestrial Gymnamoebae in Relation to Soil Depth and Precipitation Events Following a Drought in Southeastern U.S.A.

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Summary. The objectives of this research were to examine the abundance, diversity and patchiness of gymnamoebae sampled at a distance of 3.0 cm apart and at three soil depths (surface, 5.0 cm and 10.0 cm) during a drought and after a significant precipitation event. The observed gymnamoebae were categorized into four morphotypes using a standard categorization scheme, but genera of the family Cochliopodiidae were categorized separately since their abundance patterns were different from the other gymnamoebae, and taxonomically they possess characteristics of both naked and testate amoebae. Gymnamoebae abundance was significantly correlated with soil moisture ($r = 0.75$, $n = 18$, $p < 0.001$). The mean abundance of gymnamoebae (combining all the data) after the precipitation event was significantly larger than the abundance during the drought based on a univariate ANOVA test ($F(2, 15) = 12.8$, $p < 0.001$). Furthermore, the mean gymnamoebae abundance of the triplicate samples taken at the surface and at 5.0 cm after the precipitation event was significantly greater than the mean abundance during the drought $F(1, 4) = 18.3$, $p < 0.013$ (surface), $F(1, 4) = 15.0$, $p < 0.018$ (5.0 cm). There was no significant difference in the mean gymnamoebae abundances at a depth of 10 cm, $F(1, 4) = 6.4$, $p = 0.064$. Within triplicates assays, diversity coefficients were generally more similar following the precipitation event than in samples taken during the drought, and there was evidence of patchy distribution in most of the triplicate samples taken at 3.0 cm apart.

Key words: Cochliopodiidae, gymnamoeba abundance, microbial ecology, precipitation events, soil biology, testate amoebae.

INTRODUCTION

Relative to global climate issues, considerable research has been done on the importance of precipitation events in arid and semiarid regions on the ecology of macrobiota (e.g., Montana *et al*. 1995, Lin Guanghui and Ehleringer 1996, Gebauer and Ehleringer 2000), but less on terrestrial protozoa (e.g., Parker *et al.* 1984) and gymnamoebae (e.g., Rodriguez-Zaragoza and Garcia 1997, Anderson 2000) where water availability is considered the primary limiting factor.

Studies investigating how changes in precipitation patterns in arid and semiarid regions affect terrestrial gymnamoebae populations are especially important considering the ecological role gymnamoebae play in terrestrial systems including the cycling of nutrients (Stout 1980, Foissner 1997, Treonis and Lussenhop 1997), the regulation of bacterial populations (Darbyshire and Greaves 1967, Habte and Alexander 1977, Clarholm

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1981), and as a possible food source for earthworms and other soil-dwelling invertebrates (Bonkowski and Schaefer 1997, Anderson and Bohlen 1998).

Although it has been established that moisture is a significant environmental factor affecting soil gymnamoebae abundance, little is known about the effects of rain bursts on gymnamoebae abundance at varying depths especially in semi-arid or marginally arid regions in relation to global climate change. The study investigated the following research questions:

(1) What are the effects of precipitation after an extended drought on the abundance and diversity of gymnamoebae sampled at three soil depths (surface, 5 cm and 10 cm)? (2) What are the differences in abundance and diversity of gymnamoebae at the three sample depths? (3) To what extent is their evidence of patchiness in gymnamoebae abundance in three closely spaced samples (within 3 cm apart) taken at each of the three depths?

MATERIALS AND METHODS

Study location and soil characteristics

The study site was located in Americus, Georgia on the Georgia Southwestern State University Campus (33° 3' 7.79" N, and, 89° 13' 1.83" W) at *ca* 935 m above sea level. Soil samples were taken in an area of the campus shaded by pine trees and sparse grasses that had not been fertilized or cultivated for at least 30 years. A spade was used to search for and identify a loosely compressed soil with a surface horizon that continued for at least 15 cm. This kind of soil was chosen to more clearly investigate the effects of depth on gymnamoebae community structure while minimizing the effects of soil type associated with horizon changes. The sampling site was covered by a very thin layer (< 1.0 cm) of pine needles and leaf litter that were brushed away before each sampling. A homogeneous appearing surface horizon extended beyond 20 cm.

Data of measured soil characteristics and precipitation trends are shown in Table 1. Triplicate samples were prepared at the surface, 5.0 cm and 10.0 cm during an extended drought and two months later during a series of brief rain bursts. To collect soil samples, three cork borers were simultaneously inserted laterally into the soil in a 3 cm space and the cores were removed.

The sample was immediately taken to the lab in a zip-lock bag, removed and cut in half lengthwise. Half was used to determine soil characteristics and half was used for the enumeration technique (explained below). Enlarging the trench with a hand trowel and exposing fresh soil prevented contamination of later-date samples. Soil moisture was determined by measuring weight loss after drying in an oven at 100 °C and % organic matter was calculated by measuring additional weight loss after ashing the dried core at 500 °C overnight. The pH of each sample (n = 18) was determined using a pH meter (Orion SA520, Orion Research Inc. Boston, MA), and the soil temperature was recorded *in situ* by inserting a centigrade thermometer into the soil. The soil at each depth was tested once for water retention using a combination permeameter, (Gilson model HM-80, Lewis Center, OH) and precipitation data were obtained from the Plains Experimental Station, located 10 km from the study site.

Enumeration technique

Numbers of gymnamoebae/g of dry soil were determined using a laboratory culture enrichment method (Anderson and Rogerson 1995, Anderson 2000). The half of the core sample designated for enumeration was suspended in 10 ml of 0.45 µm pore size -filtered pond water in a graduated 15 ml centrifuge tube, shaken to flocculate attached amoebae and allowed to stand for a period of *ca* 0.5 min to sediment large particles. A 2 ml aliquot of the diluted suspension was removed and further diluted with 10 ml of 0.45 µm pore size filtered pond water in a 100-ml beaker. Using a micropipette, 10-µl aliquots were placed into each well of a 24-well sterile Falcon culture dish, where each well already contained 2 ml of 0.45 µm filtered pond water and a small piece (*ca* 1 mm^3) of malt-yeast agar (Page 1983) to support bacterial growth as a food for the gymnamoebae. Two culture plates were used for each sample, totaling 48 wells. The plates were wrapped in plastic film to avoid moisture loss and placed in an incubator at 19 °C for 14 to 16 days to allow proliferation of the gymnamoebae for counting and morphotype categorization. Cultures were observed with a Nikon inverted microscope using phase contrast optics, a 40x objective lens and a 15x eyepiece.

Because gymnamoebae have few definitive diagnostic characteristics at the light microscopic level, a morphotypic categorization scheme was used that has been extensively applied in recent ecological studies (e.g. Anderson and Rogerson 1995, Bischoff and Anderson 1998, Bass and Bischoff 2001). Morphotype 1 (mt-1) are amoebae with extended lobose or fine sub-pseudopodia during locomotion such as *Acanthamoeba* sp., *Mayorella* sp., *Vexillifera* sp., *Thecamoeba* sp., *Echinamoeba* sp., and *Rhizamoeba* sp. Type 2 are limax non-eruptive gymnamoebae. Genera of this type include *Hartmannella* sp., *Glaeseria* sp., and *Saccamoeba* sp. Type 3 are limax amoeba exhibiting eruptive locomotion by extrusion of a hyaline cap. Genera of type 3 include *Vahlkampfia* sp. and *Naegleria* sp. Type 4 are flattened or discoid, circular or fan-shaped, only sporadically with protruding elongated sub-pseudopodia. Representative genera of type 4 are *Platyamoeba* sp. and *Vannella* sp. Genera of the family Cochliopodiidae (Page 1988) were categorized separately because some genera are partially covered by microscales, characteristic of testate amoebae, but are surrounded by a pseudopodial base that is more typically associated with naked amoebae. Hence, they are of uncertain taxonomic status as reported by Page (1988).

The frequency of occurrence of each morphotype was tallied in the 48 wells of the culture dishes. This frequency count was converted to number of individuals per ml of original sample suspension by correcting for the dilution steps described above. The total number in the original volume of the suspension was calculated and divided by the soil sample dry weight to obtain the number/g soil for each morphotype.

Statistical analyses

Statistical data were computed using SPSS 10.0™ software. A univariate ANOVA was used to analyze the effect of precipitation on total abundance while controlling for depth as a covariate. A linear

Table 1. Mean data for soil characteristics at each sampling depth. Precipitation is expressed as millimeters of rain 14-days prior to sampling and the number in parentheses is the number of days it rained during the 14-day period

	Surface - 4/17/01		Surface - 6/21/01	
	Mean	Range	Mean	Range
% H_20	7.3	6.0 - 9.1	24.1	23.6 - 26.8
% Organic	4.8	4.0 - 5.9	8.6	4.0 - 13.0
pH	6.4	6.3 - 6.5	6.3	5.9 - 6.7
Temp °C	18	-	24	-
H_2O Retention[a]			51.1 ml/100cm^3 dry soil	
Precipitation	20 (2)	-	130 (11)	-
	5 cm Depth - 4/26/01		5 cm Depth - 7/4/01	
% H_2O	5.3	3.7 - 7.2	11.3	9.6 - 13.3
% Organic	4.9	3.3 - 7.1	4.6	2.3 - 5.7
pH	6.3	6.2 - 6.4	6.6	6.5 - 6.7
Temp °C	21	-	29	-
H_2O Retention			43.8 ml/100cm^3 dry soil	
Precipitation	13 (1)	-	144 (7)	-
	10 cm Depth - 5/12/01		10 cm Depth - 7/4/01	
% H_20	1.5	1.0 - 3.6	7.3	4.8 - 9.7
% Organic	3.0	1.2 - 5.0	2.2	1.6 - 2.7
pH	6.3	6.1 - 6.5	6.6	6.6 - 6.7
Temp °C	22	-	28	-
H_2O Retention			37.0 ml/100cm^3 dry soil	
Precipitation	25 (1)	-	144 (7)	-

[a]Water retention was measured once for each soil depth.

regression was used to calculate the correlation between abundance and soil moisture. Diversity (H) was calculated using the Shannon-Wiener formula ($H = - S\ p_i\ \log_2\ p_i$), where H is the diversity coefficient, and pi is the proportion of each type of gymnamoeba relative to the total numbers. Since it is not always possible to identify individuals to species level, morphotypic categories or "type of gymnamoebae" were determined by size (5 - µm increments), locomotion pattern, and morphology.

RESULTS

Soil characteristic data measured before and after the precipitation events are shown in Table 1. During the drought the soils at all three depths were very dry and none contained water near their water retention capacities. The surface soil had a mean moisture content of 7.3 % and the soils at 5.0 and 10.0 cm had mean moisture contents of only 5.3 and 1.5 %. After the precipitation events the mean moisture content of the surface soil was 24.1%. The mean moisture content for the soil at 5.0 cm was 11.3 %, and the mean moisture content of the soil at 10.0 cm was 7.3 %. The soils at 5.0 and 10.0 cm depth were wetter after the precipitation events, but still did not retain water near their full potential.

Precipitation events and gymnamoeba abundance

Addressing the first research question, precipitation events had a significant effect on the abundances of gymnamoebae. A univariate ANOVA with depth as a covariate showed a significant difference $F(2, 15) = 12.8$, $p < 0.001$ in the mean total abundance of gymnamoebae (combining all sampling depths) between the dry, drought condition soil and the moist, post precipitation soil. The mean total abundance for the dry soil (sampled between April 17th and May 12th) was 1114 gymnamoebae/g dry soil, and the mean total abundance for the moist soils was 4118/g. Further ANOVA tests using abundance of gymnamoebae as the dependent variable and moisture as the independent variable compared the mean differences in gymnamoebae abundances for the dry soil and the moist soil at each depth. There were significant differences in the mean gymnamoebae abundances at the surface $F(1, 4) = 18.3$,

Table 2. Abundances (numbers/g soil dry weight) and diversity coefficients for the three replicate samples (3 cm apart) at each depth before and after substantial precipitation events

				Surface sample (4/17/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
1	0	0	0	1766	0	1766	1.3
2	0	0	0	507	0	507	1.4
3	0	0	0	578	0	578	1.8
Mean	-	-	-	950	-	950	1.3
S.D	-	-	-	707	-	707	0.2
				Surface sample (6/21/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
4	0	391	391	6652	196	7630	1.8
5	417	556	0	5417	0	6390	2.7
6	229	344	0	3094	229	3896	2.5
Mean	215	430	130	5054	141	5972	2.3
S.D	209	111	225	1806	124	1901	0.5
				Soil at 5 cm (4/26/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
7	65	0	0	588	0	652	1.3
8	40	40	40	370	40	530	2.4
9	0	0	0	858	50	908	1.9
Mean	35	13	13	605	30	697	1.8
S.D.	33	23	23	244	26	192	0.5
				Soil at 5 cm (7/4/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
10	0	0	0	193	2759	2952	2.9
11	206	206	0	0	4535	4947	3.3
12	0	0	0	0	2655	2655	2.9
Mean	69	69	-	64	3316	3518	3.0
S.D	118	118	-	111	1056	1246	0.2
				Soil at 10 cm (5/12/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
13	0	0	0	284	805	1089	3.2
14	0	0	0	1107	842	1947	2.1
15	0	0	99	999	954	2052	2.7
Mean	-	-	33	797	867	1696	2.6
S.D.	-	-	57	447	77	528	0.5
				Soil at 10 cm (7/4/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
16	120	0	0	179	2453	2752	3.4
17	0	0	69	0	2260	2329	2.6
18	0	0	180	180	3780	3510	2.9
Mean	40	-	83	119	2621	2863	2.9
S.D	69	-	91	103	468	598	0.4

$p < 0.013$, and at a depth of 5.0 cm $F(1, 4) = 15.0$, $p < 0.018$. However, there was no significant difference at the 10.0 cm depth $F(1, 4) = 6.4$, $p = 0.064$. Furthermore, there was a strong correlation between total gymnamoebae abundance and moisture ($r = 0.75$, $n = 18$, $p < 0.001$). The regression equation for the correlation between abundance and moisture was $Y' = 3.022X + 2.188$.

Gymnamoebae abundance and diversity related to soil depth

The results of the second research question addressing differences in gymnamoebae abundance and diversity at the three sample depths are shown in Table 2. There was a general trend of increasing abundance with increasing depth for the dry soil samples. The mean total abundance (1696/g) of gymnamoebae in the dry soil at 10.0 cm was substantially larger than the mean abundance at the surface (950/g), and about twice as dense as the dry soil at 5.0 cm (697/g). This is particularly interesting because the soil at 10.0 cm depth on May 12th contained a mean moisture content of only 1.5 %. Mean diversity indices also increased with depth in the dry soils ranging from H = 1.3 in the soil at the surface to H = 2.6 at 10.0 cm. This was due to the presence of large numbers of individuals of the family Cochliopodiidae (Page 1988) with lengths ranging from 25-97 μm and mt-4 species (Platyamoeba and Vannella) with a mean length of 9.4 ± 3.4 μm.

By contrast, gymnamoeba density decreased with depth after the precipitation events. The mean density of gymnamoebae in the moist soils was respectively: surface soil (5972/g), 5.0 cm depth (3518/g), and 10.0 cm depth (2863/g). Unlike the dry surface soil that was completely dominated by mt-4 gymnamoebae, the moist, post-precipitation surface soil contained all four morphotypes and species in the family Cochliopodiidae. The mean diversity coefficients of the morphotypes observed in the post precipitation soils sampled at the surface, 5.0 and 10.0 cm depths were similar (H = 2.3, 3.0 and 2.9). However, the mean diversity coefficients of the samples taken at the three depths in the dry soils (H = 1.3, 1.8 and 3.0) were more varied and may indicate greater variation in community composition with depth following precipitation events.

Patchiness of gymnamoebae morphotypes

Addressing question 3, there were six sample collections where triplicate samples were taken on the same day within a horizontal distance of 3.0 cm, and there is evidence of patchy distribution for the total abundance and for each morphotype of gymnamoebae within the triplicate samples for each of the sample collections.

Evidence of patchy distribution included variations in populations of mt-4. For example, mt-4 densities in sample 1 (Table 2) were three-times larger than samples 2 and 3. The abundance of mt-4 in sample 4 is double that of sample 6, and the abundance of mt-4 in sample 9 is double that of sample 8. The total abundance of sample 11 is almost double that of samples 10 and 12 due to a localized high occurrence of Cochliopodiidae. The abundance of gymnamoebae in sample 13 is about half that of sample 14 and 15 because far fewer mt-4 were observed. Samples 16, 17 and 18 (the moist soil at 10.0 cm) showed the least amount of patchiness. There is no evidence of a relationship between patchiness and soil moisture. Thus, rain bursts while increasing overall abundances and diversity of gymnamoebae in the more moist uppermost samples had no apparent effect on the patchiness of gymnamoebae.

DISCUSSION

This report confirms findings of previous studies that protozoan abundances increase following major precipitation events (e.g. Darbyshire 1976) and gymnamoebae (e.g., Clarholm 1981, Rodriguez-Zaragoza and Garcia 1997, Anderson 2000, Bass and Bischoff 2001). This report advances our knowledge of the ecology of soil gymnamoebae by providing a more detailed analysis of the changes in abundances of morphospecies and by describing the effects of precipitation events following an extended drought. This situation more closely approximates the conditions that prevail in arid and semi-arid environments and therefore has significance in elucidating how precipitation in arid regions can affect terrestrial microbial communities and their productivity. The data, moreover, go beyond abundance by including variations in diversity and patchiness with depth in relation to precipitation events.

The drought and post precipitation gymnamoebae density data are useful in estimating how rapidly trophonts can emerge and proliferate. Based on a linear regression analysis, Anderson (2000) reported that as much as 20 % of the gymnamoebae could be active in soil with 10 % moisture, 50 % active with soil moisture near 20%, and the proportion of active amoebae could be as high as 70-80 % with soil moisture content above 25 %. However, these data were for a site in northeastern U.S.A.

and there may be significant differences for other soil types. The mean water content of all three drought soils in the current study was low ranging from 7.3 % at the surface to a very dry 1.5 % at 10.0 cm. Considering Anderson's linear regression model as a reasonable estimate of likely active stages, all three dry soils should have contained very few, if any, active gymnamoebae. Even if there was a very small proportion of trophonts present in the dry soils, it is unlikely that they would have been observed since only a 10 µl aliquot is deposited in each well of the culture plate. Hence, the enumeration process would favor the transfer of encysted forms to the culture-plate wells, thus the active gymnamoebae observed would be, with few exceptions, those individuals that excysted in the culture wells. It is reasonable to infer that the gymnamoebae densities reported in Table 2 for the dry soils were encysted in the natural environment.

The post precipitation soils were wetter and had higher gymnamoebae densities than the dry soils presumably due to the emergence and proliferation of trophozoites. At the surface the mean density of gymnamoebae increased 6-fold from 950/g during the drought (mean soil moisture 7.3 %) to 5972/g following the precipitation events (24.1 % soil moisture). The density increase at the surface following precipitation was due mostly to a burst in mt-4. This is consistent with other studies (Anderson 2000, Bass and Bischoff 2001) that reported large increases in the density of mt-4 (Vannella and Platyamoebae) with increased moisture. At 5.0 cm, the mean water content increased from 5.3 to 11.3 % following precipitation. Both of these moisture levels are low and we would expect almost complete encystations at 5.3 % moisture and only about 10 % of the gymnamoebae to be active at 11.3 %, yet the density of Cochliopodiidae increased from a mere 30/g during the drought to a mean of 3316/g following precipitation. A similar pattern of large Cochliopodiidae density increases with a small moisture increase was observed at 10.0 cm. At this depth soil moisture increased from a very dry 1.5 to 7.3 %, but the density of Cochliopodiidae tripled from a mean of 867/g to 2621/g. The cause for the high numbers of Cochliopodiidae is not clear, but perhaps the morphology of Cochliopodiidae, including the presence of microscales often surrounded by a pseudopodial base enables them to conserve cytoplasmic moisture and survive in the smallest of water films.

The significant differences in total mean abundance for the post precipitation soils, and the strong correlation between soil moisture and abundance support the conclusion that the gymnamoebae in this soil possessed a tenacious capacity to proliferate when environmental conditions improved. Although no significant difference in total mean abundance was found for the gymnamoebae community at 10.0 cm comparing the drought samples to those after the precipitation events, the total mean gymnamoebae abundance at that depth increased by 60 %. The capacity of soil gymnamoebae to withstand drought conditions and proliferate soon after precipitation events is encouraging.

Overall, the mean density of gymnamoebae in the wet soil was 4 times greater than the dry soil. Similarly, Anderson (2000) conducted a four-year study in Northeastern U.S.A and reported mean gymnamoebae abundances were four times larger during the warm and wet El Niño winter of 1997-1988 than the mean of the non El Niño years, and Rodriguez-Zaragoza and Garcia (1997) reported a doubling of gymnamoebae densities following precipitation in a Mexican desert at depths of both 10 and 30 cm.

The trend of increased gymnamoebae abundance and diversity following precipitation was more extensive at the surface than at depth. For example, the mean density of gymnamoebae in the wet surface soil was six times greater than the dry soil and the drier soil contained only mt-4 species (*Platyamoeba* and *Vannella*). After a substantial series of rain events, all four morphotypes and genera of the family Cochliopodiidae (most likely *Cochliopodium bilimbosum*) were observed in the moist surface samples demonstrating the capacity of soil gymnamoebae to flourish, probably due to excystment and proliferation when conditions improved.

An interesting pattern in the soil at 5-cm was the substantial increase in the abundance of Cochliopodiidae and the concomitant decline of mt-4 gymnamoebae following the rain events. The data suggest the possibility of a predator prey relationship, or the ability of Cochliopodiidae to competitively exclude mt-4 in the microbial community. This observation needs verification and could be an interesting future study.

At 10-cm depth, mt-4 gymnamoebae and Cochliopodiidae dominated the drier soil. Only sample 15 contained Vahlkampfiid-type, mt-3 gymnamoebae and these were present in relatively small densities. Even though 144 mm of precipitation occurred during the 14-days preceding sampling, the soil moisture only increased from a very dry 1.5 % to 7.3 %. This increase in moisture, however small, seemed sufficient to triple the mean abundance of Cochliopodiidae and drive down the populations of mt-4, following a pattern very similar

to the data from soil at 5.0 cm. Additionally, mt-1 which were not detectable in the drier soil at 10.0 cm were found in sample 16, and mt-3 abundances increased slightly.

There was evidence of patchy abundance patterns in almost all of the triplicate samples (Table 2). Surface samples 4, 5 and 6 were the wettest soils sampled and they are the best data to look for patterns of reduced patchiness with increased moisture. The abundance patterns of mt-2 display only a small amount of patchiness and that could be attributed to error in the sampling method and not true patchiness in the soils. There is, however, evidence of patchy abundance patterns for morphotypes 1, 3, 4 and Cochliopodiidae in samples 4, 5 and 6.

With some of the triplicate data points, two of the assays yielded similar abundance points and a third identified either a growth hot spot, like sample 11 or a cold spot, like sample 13 where mt-4 abundances were low compared to samples 14 and 15. This patchy pattern of spatial distribution across a space of only 3.0 cm is of interest, but the causes are not known. It could occur due to heterogeneous distribution of prey, variations in nutrient concentrations, unevenness in moisture, varied physical characteristics of the soil, etc.

Overall, the data demonstrating the remarkable capacity of soil gymnamoebae to withstand severe drought and resume their role in the microbial food web in response to a series of brief rain bursts are reassuring. More research integrating our knowledge of gymnamoebae ecology with global climate issues is needed so that we may more fully understand the dynamics of our changing environment and its likely influence on terrestrial microbial communities.

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